**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| In re application of<br>Hofstraat et al. | |
| Application Serial No.:<br>09/380,336 | Attorney Docket No.: DVME-1009<br>Group Art Unit: 1641 |
| Filed: November 23, 1999 | Examiner: G. Gabel |
| For: DIAGNOSTIC NEODYMIUM (III) YTTERBIUM (III) OR ERBIUM (III) ION-LIGAND COMPLEXES | |

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**RULE 132 DECLARATION**

Assistant Commissioner for Patents
BOX AF
Washington, D.C. 20231

I, Klemens Brunner hereby declare:

1. I, Klemens Brunner, graduated from Vienna University with a Ph.D., and I am an expert in the area of photoluminescence. I'm currently working in the polymers and organic chemistry department of Philips National Labs, the Netherlands in the field of luminescent organic materials. I have read U.S. Patent Application serial No. 09/380, 336: "Diagnostic Neodymium (III), Ytterbium (III) or Erbium (III) Ion-Ligand Complexes" by Hofstraat et al. (hereafter "the application,") and I am generally familiar with the state of art in the research area concerned.

2. I have fully understood the matter described in the Application and have also studied U.S. Patent No. 5,830,769 to Wieder et al. (hereafter "Wieder et al.") and U.S. patent No. 6,159, 686 to Kardos et al. (hereafter "Kardos et al."), which have been cited in the Office of Action dated August 3, 2001.

3. Wieder et al. teaches several photoluminescence assay embodiments only two of which are of possible interest with respect to the application, whose mechanisms are best illustrated by Figures 1 and 2 below, respectively. The first assay embodiment employs a fluorescent ligand-lanthanide metal complex that uses substituted pyridine or polyamine polycarboxylic acid as the ligand, which absorbs light in the ultraviolet (hereafter "UV") range (< 400 nm).

4. The ligand used in the first embodiment of Wieder et al does not contain a sensitizing moiety which absorbs light in the 400-1000 nm region. The first assay embodiment employs rhodamines or a fluorescein type material to quench the fluorescence emission from the ligand-lanthanide metal complex when the rhodamines or fluorescein type material is brought in close proximity (about 30.6Å, see col. 9, lines 55-60 of Wieder et al.) to the ligand-lanthanide metal complex via an antibody-analyte binding mechanism. In contrast, the present invention employs a ligand-lanthanide metal complex, wherein the ligand itself contains a sensitizing moiety such as rhodamines or fluorescein. As a result, the distance between the sensitizing moiety and the lanthanide moiety in the ligand-lanthanide metal complex of the present invention is short enough to enable the direct transfer of energy from the sensitizing moiety to the lanthanide metal via a "S-T-M" route. The mechanism with regards to the "S-T-M" route is discussed at col. 12, lines 52-56 of Wieder et al. and will be further discussed hereafter.

5. To further clarify the fundamental difference between Wieder et al. and the present invention, Figure 1 illustrates the mechanism of the first embodiment, the "quenching" embodiment, of Wieder et al. In this embodiment, the ligand, e.g., substituted pyridine and polyamine polycarboxylic acid, which has an excitation wavelength in the UV range, of a fluorescent ligand-lanthanide metal complex is excited to a singlet state by a UV light source. Upon excitation, the ligand of the ligand-lanthanide metal complex decays

to a triplet state and transfers its energy to the lanthanide metal of the lanthanide metal complex, which is further quenched by a fluorescence quenching material ("quencher") such as a Period IV transition metal complex, rhodamine, or a substituted flurorescein. During this quenching process, the quencher absorbs energy from the excited lanthanide metal. The quencher then decays back to the ground state by emitting a red light (about 600-700 nm wavelength).

6. In this embodiment, the measured output is e.g. the amount of quenching detected by a detector. In other words, the measurement is realized by measuring the "absence" of fluorescence emission from the lanthanide of the ligand-lanthanide metal complex in the wavelength range of fluorescence emission of the lanthanide. In this particular embodiment, the wavelength range of fluorescence emission of the lanthanide in the lanthanide metal complex is in the visible light range. Therefore, the detector used to detect the "absence" of the fluorescence emission of the lanthanide metal has a detection wavelength range of green – red visible light.

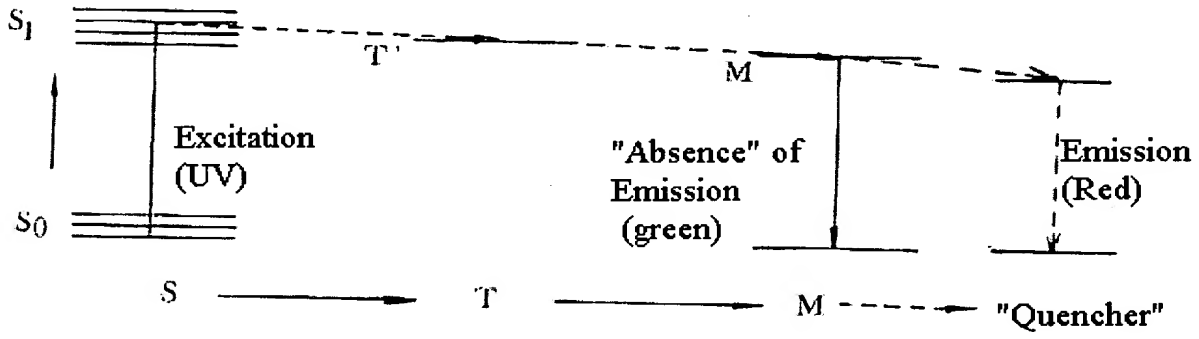



FIG. 1

7. The second assay embodiment of Wieder et al. employs a fluorescent ligand-lanthanide metal complex that uses substituted pyridine or polyamine polycarboxylic acid as the ligand, which absorbs light in the UV range. The second assay embodiment employs a substituted diphenyloxazole (see col. 13, table 1 of Wieder et al.) to enhance the fluorescence emission from the ligand-lanthanide metal complex when the substituted diphenyloxazole is brought in close proximity (about 50Å, see col. 13, lines 2-5 of

Wieder et al.) to the ligand-lanthanide metal complex via an antibody-analyte binding mechanism. The substituted diphenyoxazole also absorbs light in the UV range. The second assay embodiment of Wieder et al. does not employ a material containing rhodamines or fluorescein type moieties. The second assay embodiment of Wieder et al. also does not employ a ligand containing a sensitizing moiety which absorbs light in the 400-1000 nm region.

8. The second embodiment taught by Wieder et al. is illustrated in Figure 2, wherein an enhancer, such as substituted diphenyloxazole (see col. 13, table 1 of Wieder et al.) which has an excitation wavelength in the UV range, is activated and excited to a singlet state by UV light. The enhancer is in close proximity (about 50Å, see col. 13, lines 2-5 of Wieder et al.) to the ligand of a ligand-lanthanide metal complex. After excitation, the enhancer transfers its energy to the ligand of the ligand-lanthanide metal complex and thereby excites the ligand to a singlet state. The ligand then decays from the singlet state to a triplet state, which results in the transfer of the energy to the lanthanide metal in the ligand-lanthanide metal complex. Finally, the lanthanide metal releases the energy by emitting a e.g. green light. In this particular embodiment, the fluorescence emissions being detected are those emitted by the lanthanide metal, which are generally in the wavelength range of visible green light. Therefore, the detector used to detect the fluorescence emissions of the lanthanide metal has a detection wavelength range of green - red visible light.

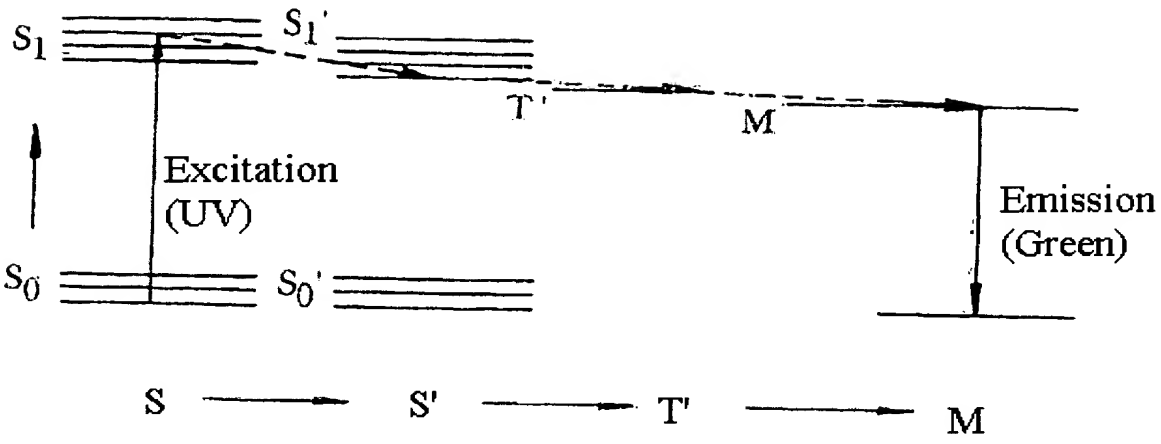



FIG. 2

9. Kardos et al. teaches a photoluminescence assay using a two-photon excitation process. In this assay, a probe such as an antibody is labeled with an up-converting reporter such as an inorganic or organic up-converting fluorescence emitting material. The probe is then contacted with particular analytes followed by exciting the up-converting fluorescence emitting material with an intense near-IR light source. The excited up-converting fluorescence emitting material then emits an output emission in the visible wavelength region and thereby returns to the ground state. A detector detects the emission in the visible light wavelength and determines whether particular analytes are present.

10. The up-converting fluorescence emitting material used in Kardos et al. can be categorized into two categories: inorganic up converting fluorescence emitting materials and organic up-converting fluorescence emitting materials. Exemplary inorganic up-converting fluorescence emitting materials are $Na(Y_xYb_yEr_z)F_4$ (phosphor particles) (cols. 15 and 16 of Kardos et al.), which is a lanthanide-containing material or lanthanide chelates though the usage of these lanthanide chelates is not actually demonstrated here. Exemplary organic up-converting fluorescence emitting materials are rhodamines (col. 30, lines 44-46). The assay of Kardos et al. generally employs one of these up-converting

fluorescence emitting materials. The inorganic phosphor particles such as those containing lanthanide are heterogeneous particles (see col. 17, lines 23 – 41 of Kardos et al.).

11. Even though it is possible to use both the organic and inorganic fluorescence emitting materials in one assay (see col. 12, lines 60-62 of Kardos et al.), using two fluorescence emitting materials as up-converting material does not provide any additional benefit to the assay. Should an organic up-converting fluorescence emitting material be used together with an inorganic up-converting fluorescence emitting material, these two materials would function independently. The lanthanide phosphor particles and the organic fluorescence emitting material disclosed in Kardos et al. will not form a complex when they are combined in a mixture because the lanthanide contained in the inorganic up-converting fluorescence emitting material is heterogeneous and is therefore incapable of binding to another ligand to form a complex. In addition, using more than one up-converting fluorescence emitting material in one assay will complicate the emission spectrum of the assay and make the analysis more difficult because each up-converting fluorescence emitting material in the assay will emit its own emission.

12. The process of exciting the up-converting fluorescence emitting material of Kardos et al. is a two-photon process as illustrated in Figures 3A-3C below. As is well known to a skilled person, a two-photon excitation process is an extremely inefficient process (the quantum yield being extremely low). Therefore, an intense near-IR light source such as those described in Kardos et al. (col. 15, lines 56-64 of Kardos et al.) is required to achieve useful detection results. In addition, the emission wavelength emitted by the fluorescence emitting material is generally shorter than the excitation wavelength used to

excite the up-converting fluorescence emitting material.

FIG. 3A

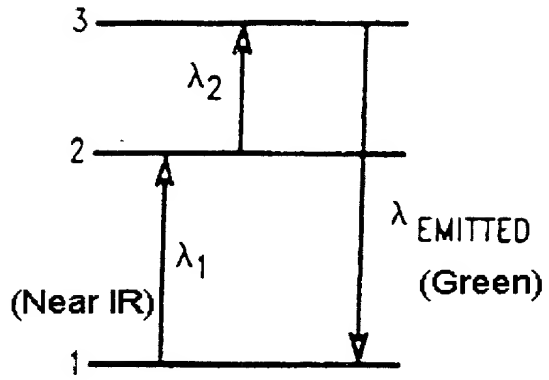



FIG. 3B

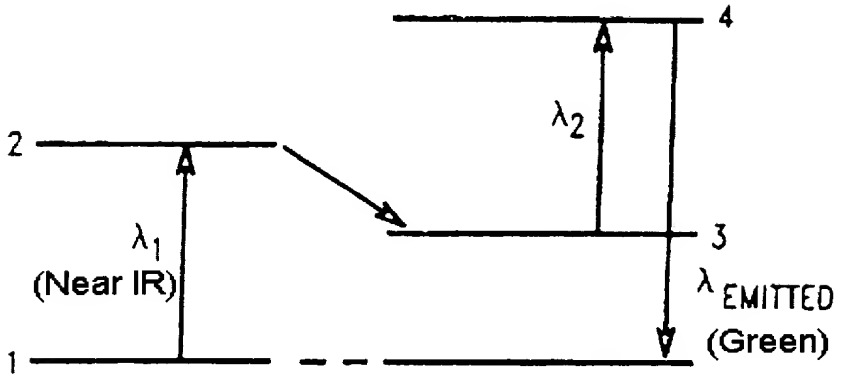
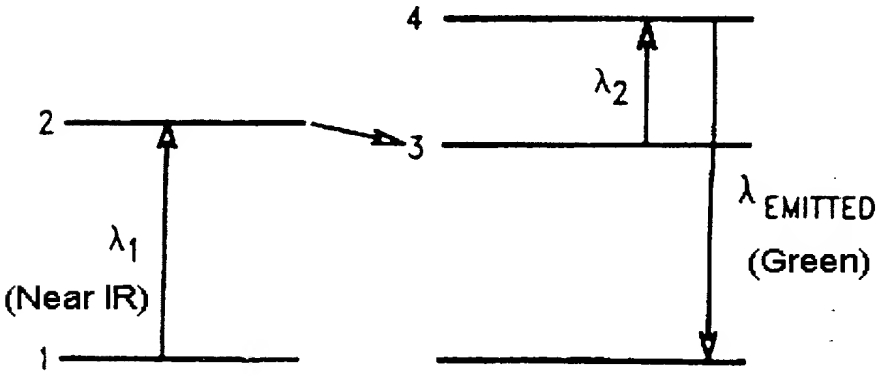



FIG. 3C




13. In contrast, the sensitizing moiety, such as a rhodamine moiety, used in the present invention is an integral part of the lanthanide complex. The sensitizing moiety absorbs

radiation and is excited to a singlet state. The sensitizing moiety then decays to a triplet state to transfer its energy to the lanthanide metal since the sensitizing moiety is a part of the ligand complexing to the lanthanide metal and, thus, in much closer proximity to the lanthanide than those taught by Wieder et al. The lanthanide metal receives the energy and emits a fluorescence emission in the near IR wavelength range, which is detected by a detector having a near IR detection range. The present invention uses a single photon excitation process as shown in Figure 4 below.

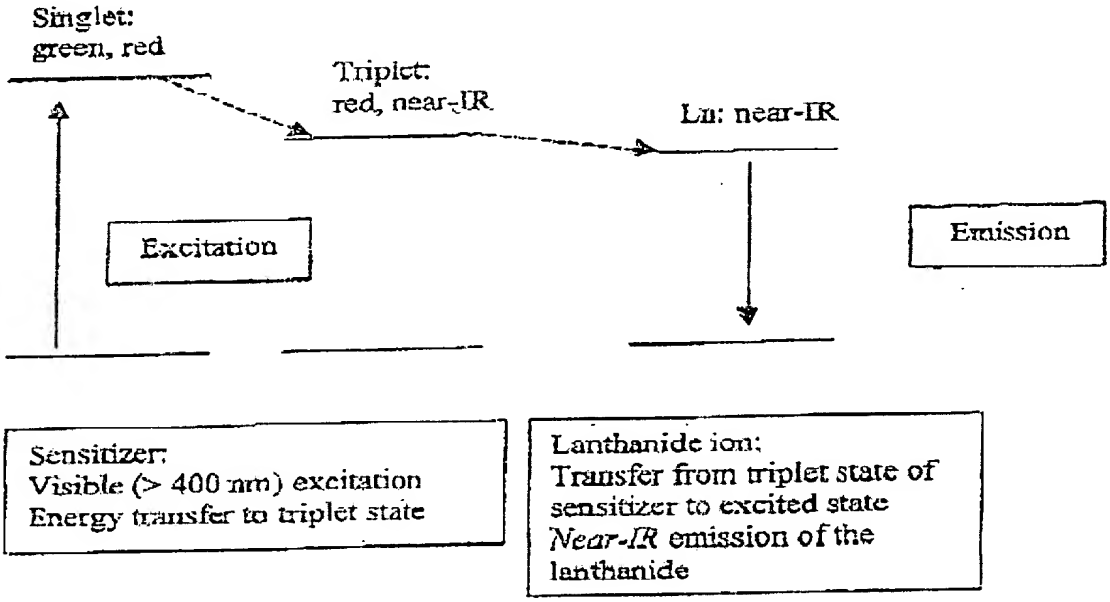



FIG. 4

14. Since the ligand used in the present invention has an excitation wavelength in the visible light range and the ligand transfers its absorbed energy directly to the lanthanide, the lanthanide complex of the present invention can be excited by a visible light source, instead of a UV light source as required by Wieder et al.

15. In contrast to the requirement of a high intensity light source due to the low excitation efficiency of Kardos et al., the present invention requires a visible light source to achieve good detection results because the single photon excitation process employed in the

present invention has a much higher excitation efficiency than the process of Kardos et al.

16. Furthermore, a person of ordinary skill in the art would not combine the teachings of Wieder et al. and Kardos et al. Even though Wieder et al. discloses dyes/sensitizing moieties (used as the "quencher") which inherently absorb in the 400-1000 nm range, when the ligand-lanthanide complex of Wieder et al. is irradiated with a 400-1000 nm light source such as is taught in Kardos et al., the ligand-lanthanide complex of Wieder et al. will not emit an IR emission that can be detected by a detector such as that employed in Kardos et al. That is because the dyes/sensitizing moieties cannot transfer their energy to the lanthanide metal complex because of the type of ligand-lanthanide metal complex employed in Wieder et al. For example, the lanthanide metal in Wieder et al. binds with ligands such as substituted pyridine or polyamine-polycarboxylic acid (see cols. 5 and 6 of Wieder et al.), which do not absorb light in the 400-1000 nm range, and the dyes/sensitizing moieties used in Wieder et al. situate themselves a substantial distance from the lanthanide metal (i.e., about 30.6A, see col. 9, lines 44-49). In fact, the dyes/sensitizing moieties of Wieder et al. which absorb in the 400-1000 nm range are used to quench the emission from the lanthanide metal complex instead of exciting it as would be the case in the present invention. For these reasons, a person of ordinary skill in the art would not combine the teachings of Wieder et al. with the teaching of Kardos et al.

17. In summary, Wieder et al. and Kardos et al. differ from the present invention in that none of them uses a ligand-lanthanide complex, wherein the ligand containing a sensitizing moiety which absorbs light in the 400-1000 nm range. Wieder et al. employs a ligand-lanthanide complex, wherein the ligand does not contain a sensitizing moiety absorbing in the 400-1000 nm range.

18. I declare that all statements made herein that are of my own knowledge are true and that all statements that are made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title

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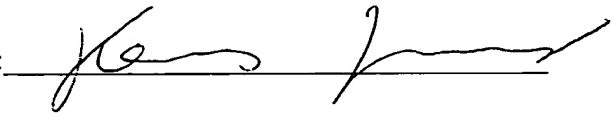
Signed: 

Date: 9 July 2002